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Adsorption of Enteroviruses to Soil Cores and Their Subsequent Elution by Artificial Rainwater

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16. ABSTRACT
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Adsorption of Enteroviruses to Soil Cores and Their Subsequent Elution by Artificial Rainwater

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The adsorption and elution of a variety of human enteroviruses in a highly permeable, sandy soil was studied by using cores (43 by 125 mm) collected from an operating recharge basin on Long Island. Viruses studied included field and reference strains of polioviruses types 1 and 3 and reference strains of coxsackie virus B3 and echovirus types 1 and 6. Viruses suspended in treated sewage effluent were allowed to percolate through soil cores, and the filtrate was assayed for unadsorbed viruses. To determine the likelihood of desorption and mobilization, soil-bound viruses were subjected to a rinse with either treated sewage effluent or simulated rainwater which reflected the anion, cation, and pH characteristics of a typical northeastern United States rainfall. The results demonstrated that all polioviruses tested, including both reference and field strains, adsorbed extremely well to cores. Adsorption was somewhat reduced when clean, unconditioned soils were used. Soil-bound poliovirus strain LSc was not significantly mobilized by flooding columns with either a sewage effluent or rainwater rinse. One virus was mobilized by both types of rinses. The amount of viruses mobilized by rainwater rinses ranged from 24 to 66%. Variable adsorption-elution results were observed with other enteroviruses. Two guanidine-resistant mutants of poliovirus LSc demonstrated a soil adsorption-elution profile different from that of the parent strain. The data support the conclusion that soil adsorption-elution behavior is strain dependent and that poliovirus, particularly strain LSc, represents an inappropriate model.

The movement of human viruses through soils is an important consideration in the design of wastewater recharge systems. Virus mobility within soil has been shown to be influenced by the nature of the virus, the composition of the soil, the ionic strength of the applied wastewater, and the rate and schedule of wastewater application (2, 13). Although numerous studies have addressed the likelihood of virus movement in soils (reviewed by Gerba et al. [7]), few can be used to accurately predict this phenomenon. The chief constraints on a majority of these studies have been: (i) the limited number of virus types studied (particularly the nearly exclusive use of poliovirus type 1 LSc 2ab); (ii) the use of packed laboratory columns; and (iii) the use of fluids of nonsewage origin for virus-suspending medium and the conditioning of columns.

Recently, Gerba and Goyal (5) reported the adsorption of 27 different enteroviruses to 9 different soil types, using a 30-min batch adsorption procedure. The authors observed that all three types of polioviruses, including reference and field isolates, adsorbed extremely well to a majority of the test soils. Other enteroviruses (coxsackie- and echoviruses) demonstrated varied adsorption patterns depending on the origin of the virus. Reference strains of coxsackievirus (B1 through B6) were found to readily adsorb to soils, whereas natural isolates of coxsackievirus B4 adsorbed poorly. Adsorption of natural coxsackievirus strains to Flushing Meadow soil was greatly enhanced by the presence of 10 mM Ca^{2+}. The adsorption of both reference and field-isolated echoviruses was found to be greatly dependent on the particular virus strain. Reference strains of echovirus types 1 and 29 were poor adsorbers, whereas the remaining echovirus types tested showed good adsorption. Natural isolates of echovirus type 1 failed to efficiently adsorb to most soils. The authors concluded that the adsorption of virus to soils was indeed strain dependent in batch experiments.

Once adsorbed, viruses are not irreversibly bound to soil particles and may desorb and move through soil under certain conditions. Conductivity of the percolating solution appears to play an important role in desorption and mobilization of virus. This was first observed by Wellings et

al. (23) after the isolation of enterovirus from groundwater beneath a recharge basin after a period of heavy rainfall. Mobilization of adsorbed viruses by low-ionic-strength fluids was also reported by Lance et al. (13) and Gerba and Lance (6), using packed and conditioned columns (10 by 250 cm). These authors observed the movement of poliovirus type 1 (LSc 2ab) to greater column depths after the application of deionized water, simulating a burst of rainfall. Desorbed viruses were found to readsorb at lower levels in the column, with few being detected in the column outflow. In addition, viral desorption and movement was greatly reduced or eliminated by adding salts, by immediately applying sewage effluent, or by drying the column for 3 to 5 days before application of deionized water. Duboise et al. (3), studying the removal efficiency of poliovirus type 1 (Chat) in nonsterile natural forest soils, also observed virus mobilization after using low-ionic-strength fluids. The authors demonstrated that peak viral elution from soil cores (19.5 by 5.6 cm) coincided with the minimum column effluent conductivity, brought about by distilled water rinses.

In a recent field study conducted on Long Island, this laboratory isolated several different enterovirus strains from glacial aquifers beneath wastewater recharge basins (20–22). The failure to recover certain enteroviruses, including poliovirus, suggested that the local soils may have exhibited a preferential binding for certain virus types. The purpose of the present study was to determine both the virus-binding capacities of Long Island soil, using natural cores from operating recharge basins, and the extent of virus desorption by rainwater. The study made use of a variety of enterovirus field isolates and laboratory reference strains in an effort to delineate the differences in adsorption-desorption characteristics among related strains of enterovirus.

MATERIALS AND METHODS

Virus. Poliovirus type 1 LSc 2ab was a gift of R. Reed (Carborundum Corp.). Poliovirus type 3 (Leon), echovirus type 1 (Farouk), and coxsackievirus B3 (Nancy) were all obtained from the National Institute of Allergy and Infectious Diseases. Echovirus type 6 (D'Amori) was purchased from the American Type Culture Collection (ATCC VR-36). All viruses were grown on monolayers of low-passage Buffalo green monkey kidney cells (BGM; Microbiological Associates) and purified to yield monodispersed cultures as described by Jakubowski et al. (10). Poliovirus type 1 (V234-1) and type 3 (V153-1 and V201-4) were isolated from secondarily treated wastewater effluents (22). Viruses were plaque purified three times on BGM and identified by serum neutralization techniques, using enterovirus-typing pools (17). Poliovirus LScg'-B (guanidine resistant) was a spontaneous mutant of strain LSc 2ab selected by growth in guanidine-containing media according to procedures described by Melnick et al. (16). The mutant was obtained by three serial passages in liquid culture at increasing concentrations of guanidine (30, 50, and 100 μg/ml). After plaquing on a guanidine-containing medium (100 μg/ml), isolates were plaque purified three times and a stock was prepared as previously described. Poliovirus type 1 LScg'-T was kindly provided by D. R. Tershak (19).

Temperature marker analysis. The virulence of the natural poliovirus isolates used in experiments was determined by temperature marker analysis (15). Plaque assays at high temperatures were performed by submersing overlay flasks, wrapped in plastic bags, in a water bath (Magni-Whirl, Blue M Electric Co.) at 39.5°C. The temperature inside the plastic bags was monitored by a Digitec 5810 thermometer and continually recorded. Temperature fluctuations never exceeded 0.3°C. Virulence was determined by calculating the efficiency of plating (EOP; determined as plaque-forming units [PFU]/ml$^{39.5}$ divided by PFU/ml^{37}). Viruses exhibiting a log EOP of >-2.0 were considered virulent strains; those with a log EOP of -2.1 to -4.0 were considered intermediate; and those with a log EOP of ≤ -4.0 were considered attenuated (25).

Cells and assay. All assays were carried out on monolayers of low-passaged BGM cells grown in minimum essential medium with Earle balance salt solution supplemented with 10% fetal calf serum, glutamine (2 mM), and antibiotics. Sample preparation, inoculation, and overlay methods have been previously described (14).

Column experiments. Cores (43 by 125 mm) used in column experiments were collected from the surface of an operating recharge basin in Medford, N.Y., using Lexan plastic tubing (43 by 250 mm). The soil of the basin, which has been described previously (21), consists mainly of sand (67%) and gravel (32%), with little clay and silt (<1%). The pH of the soil was 7.14 in 10 mM $CaCl_2$ and 7.65 in water. For each experiment, cores were matched in pairs according to similar infiltration rates as measured by percolation of 100 ml of tertiary sewage effluent through the core. Infiltration rates for all cores used throughout the study were rapid, averaging 83 cm/h. The virus-adsorbing ability of the soil was determined by challenging each of two columns with 200 ml of nonsterile, virus-containing tertiary sewage effluent. Sewage effluents were monitored to ensure that no residual chlorine was present. Column filtrates were collected and assayed for total virus PFU. Mobilization of adsorbed viruses in response to various treatments was determined by challenging one core of each pair with 200 ml of sewage effluent while rinsing the second core with 200 ml of artificial rainwater. Column effluents were collected and analyzed as above. The characteristics and chemical composition of the typical sewage effluent and the artificial rainwater used in the experiments are listed in Table 1. The rainwater, kindly provided by N. Gmur of Brookhaven National Laboratory, reflects the anion and cation contents of rainfall in the northeastern United States (4). Final column elution was accomplished by mixing the entire column in 200 ml of 3% beef extract (pH 9.5) for 5 min on a rotary shaker. After centrifugation at 5,000 rpm for 10 min, the

supernatant was decanted, neutralized, and assayed for virus PFU.

RESULTS

Virus adsorption to packed columns and natural cores. There are a number of techniques available for studying the adsorption of viruses to soils, including batch adsorption (5), packed columns (9), and natural soil cores (3). To closely duplicate natural systems, we chose from the latter two methods in determining the optimal adsorption efficiency of viruses to Long Island soils. A series of columns (43 by 125 mm) was prepared, using clean soil from the banks of a sewage recharge basin. Since preliminary experiments had indicated poor viral adsorption to clean, unused Long Island soils, columns were conditioned by passing 15 liters of unchlorinated, secondarily treated sewage through each column. Natural cores were collected from the floor of the same operating recharge basins as soil plugs (43 by 125 mm). Columns were challenged with reference strains of either poliovirus type 1 (LSc 2ab) or coxsackievirus B3. Column effluents were collected and analyzed for total PFU. The results listed in Table 2 represent the average of at least two experiments. Both reference strains of polio- and coxsackieviruses adsorbed efficiently to natural cores (94 and 98%, respectively), whereas adsorption to packed, conditioned columns in both cases was somewhat decreased (76 and 64%, respectively). These data indicated that the continual exposure of soils to sewage effluent enhanced their ability to adsorb these viruses. Based on these results, natural cores were used in all subsequent experiments for optimal enterovirus adsorption.

TABLE 1. *Chemical characteristics of tertiary treated effluent and artificial rainwater*

Determination[a]	Sewage effluent	Artificial rainwater[b]
pH	7.47 (7.05–8.34)[c]	4.4 (4.3–4.6)
Turbidity, NTU[d]	4.10 (1.0–12.5)	1.0
Conductivity, μmho	606.00 (444–680)	40.0
Total alkalinity	95.38 (48–115)	NT[e]
Total suspended solids	5.00 (1–14)	NT
Total organic carbon	15.13 (10–29)	NT
Chloride	54.13 (47–64)	2.70
Sulfate	35.63 (28–54)	5.00
Nitrate	3.38 (0.07–16.0)	0.80
Nitrite	0.11 (0.006–0.49)	NT
Ammonia	5.34 (0.06–15)	0.40
Total Kjeldahl-N	6.63 (0.90–17)	NT
Orthophosphate	6.13 (4.8–6.8)	NT
Iron	<0.05	0.010
Manganese	0.02 (0.01–0.03)	0.020
Magnesium	4.53 (2.7–7.8)	0.17
Calcium	19.00 (15–24)	0.506
Sodium	58.25 (54–68)	1.20
Potassium	12.25 (11–13)	0.20
Lead	NT	0.05
Zinc	NT	0.10
Nickel	NT	0.10
Copper	NT	0.02
Cadmium	NT	0.02
Fluoride	NT	0.10

[a] Except for pH and where noted, all determinations are in milligrams per liter.
[b] Taken from Evans et al. (4).
[c] Values in parentheses are ranges.
[d] NTU, Nephelometric turbidity units.
[e] NT, Not tested.

Adsorption and rainwater elution of field and reference poliovirus. Recent evidence has indicated that poliovirus LSc adsorbs extremely well to many soil types under a wide range of conditions and therefore may not represent a good model for the adsorption characteristics of other enteroviruses (5, 22). This hypothesis was tested by determining the adsorption profiles of a number of polioviruses, including reference and field strains, to Long Island soils. Reference strains chosen for studies included poliovirus type 1 LSc 2ab and type 3 Leon (ATCC VR-62). Field strains, isolated from treated sewage effluent during a previous study (22), included polioviruses type 1 (V234-1) and type 3 (V153-1 and 201-4). The latter two strains were found to exhibit high EOP at elevated temperatures (39.5°C) and were classified as wild or virulent poliovirus (Table 3). In addition to determining the adsorption patterns of these viruses, the mobilization of bound viruses by artificial rainwater was measured. All previous mobilization studies had been performed with distilled water. Table 4 illustrates the results of a series of experiments on the adsorption and mobilization capacities of poliovirus type 1 LSc 2ab and its field counterpart, V234-1. Both the reference strain, LSc 2ab, and the type 1 field isolate adsorbed well to cores, ranging from 93 to 96% and 82 to 89%, respectively. Although the adsorption characteristics of both virus types were the same, their elution or mobilization patterns with sewage effluent or artificial rainwater rinses were quite different. Few LSc viruses were released with either a sewage (1.37% average) or a rainwater (2.83%) rinse. Substantially more field poliovirus type 1 was released from cores by rinses with either substance, with an average of 14% being eluted with sewage effluent and 33% eluted with simulated rainwater.

The adsorption patterns of both reference strain poliovirus type 3 (Leon) and field poliovirus type 3 strains V153-1 and V201-4 were similar to that of poliovirus type 1, with over 97% of the viruses adsorbing to soil cores (Table 5). Whereas sewage effluent rinses did not appreciably remove either type 3 reference or field strains from the cores, a rainwater rinse resulted in the mobilization and elution of both the ref-

TABLE 2. *Comparison of the adsorptive capacity of poliovirus LSc and coxsackievirus B3 (Nancy) to packed conditioned columns and natural cores*

Virus type	Column type	Total PFU recovered as:[a]		
		Input (A)	Unadsorbed (B)	Theoretical adsorbed (A-B)
Poliovirus 1 LSc 2ab	Packed	3.78×10^5 (100)[b]	8.89×10^4 (23.54)	2.89×10^5 (76.46)
	Natural core	2.85×10^6 (100)	1.73×10^5 (6.97)	2.68×10^6 (94.03)
Coxackievirus B3	Packed	1.04×10^5 (100)	3.65×10^4 (35.10)	6.75×10^5 (64.90)
	Natural core	6.97×10^6 (100)	6.46×10^3 (1.01)	6.90×10^6 (98.99)

[a] Values represent the average of at least two experiments.
[b] Numbers in parentheses represent percentages of the input value.

TABLE 3. *Virulence of poliovirus type 3 field isolates*

Poliovirus strain	Titer (pfu/ml)		EOP (A ÷ B)	Log EOP
	39.5°C (A)	37°C (B)		
LSc 2ab (control)	1.00×10^7	1.30×10^7	7.69×10^{-8}	−6.8859
V153-1	1.20×10^6	7.80×10^6	1.5×10^{-1}	−1.1761
V201-4	3.40×10^5	7.40×10^5	4.5×10^{-1}	−1.6532

TABLE 4. *Adsorption-elution profiles of field and laboratory poliovirus type 1 in natural soil cores*

Poliovirus strain	Total PFU recovered from:				
	Input (A)	Unadsorbed (B)	Theoretical adsorbed (A-B)	Sewage filtrate	Rainwater filtrate
LSc 2ab	2.85×10^6 (100)[a]	1.73×10^5 (6.07)[a]	2.68×10^6 (93.93)[a]	3.68×10^4 (1.37)[b]	—[c]
	2.85×10^6 (100)	1.06×10^5 (3.72)	2.74×10^6 (96.28)	—	7.76×10^4 (2.83)[b]
V234-1	7.26×10^7 (100)	1.28×10^7 (17.63)	5.98×10^7 (82.37)	8.34×10^6 (13.94)	—
	7.26×10^7 (100)	7.64×10^6 (10.51)	6.49×10^7 (89.49)	—	2.15×10^7 (33.13)

[a] Values in parentheses are percent recoveries based on input.
[b] Values in parentheses are percent recoveries based on the theoretical adsorbed virus.
[c] —, No sample.

TABLE 5. *Adsorption-elution profile of laboratory and field strains of poliovirus type 3 in natural soil cores*

Poliovirus strain	Total PFU recovered from:				
	Input (A)	Unadsorbed (B)	Theoretical adsorbed (A-B)	Sewage filtrate	Rainwater filtrate
VR-62 (Leon)	5.85×10^6	9.35×10^4 (1.60)[a]	5.76×10^6 (98.40)[a]	1.11×10^4 (0.19)[b]	—[c]
	5.85×10^6	9.50×10^4 (1.62)	5.75×10^6 (98.38)	—	2.33×10^6 (40.52)[b]
V201-4	7.57×10^6	1.06×10^5 (1.40)	7.46×10^6 (98.60)	7.92×10^5 (1.06)	—
	6.60×10^6	7.99×10^4 (1.21)	6.52×10^6 (98.79)	—	1.57×10^6 (24.08)
V153-1	2.28×10^7	4.35×10^5 (1.90)	2.23×10^7 (98.10)	8.92×10^4 (0.40)	—
	2.28×10^7	6.64×10^5 (2.91)	2.21×10^7 (97.09)	—	1.47×10^7 (66.51)

[a-c] See Table 4.

erence strain, type 3 Leon, and the field isolates. An average of 66% of V153-1 were released from the soil during rainwater elution.

As previously illustrated (Table 1), simulated rainwater used in all experiments has an ambient pH of 4.3 and a conductivity of 40 μmhos, whereas the treated sewage effluent rinses had an average pH of 7.47 and a conductivity of 606 μmhos. Since both pH and conductivity are known to affect the mobilization of virus in soil (7), it was necessary to determine which factor played the key role in the movement of poliovirus in soil cores. Poliovirus type 3 field strain V153-1 was chosen for these experiments and was adsorbed into cores as previously described. Matching cores were eluted with either the described sewage effluent rinse, a rainwater rinse, or a rainwater rinse adjusted to pH 6.7. pH

adjustments resulted in an increase in the conductivity to 300 μmhos. Core filtrates were collected and analyzed for total PFU (Table 6). As previously indicated, the sewage effluent rinse failed to mobilize any viruses. Rinsing with normal rainwater resulted in the removal of 27% of the adsorbed viruses. Elution with higher-pH rainwater of intermediate conductivity resulted in mobilization of about 20% of the viruses. These data appeared to indicate that conductivity was a more important factor than pH in the release of V153-1 from soil cores.

Adsorption and elution of reference echovirus. To determine whether the varied patterns of adsorption, sewage, and rainwater elution observed in poliovirus occurred in other enteroviruses, similar experiments using reference echovirus were conducted (Table 7). Echovirus 1 exhibited the greatest soil affinity of all enteroviruses tested, with over 99% of the virus adsorbing and no viruses eluted with either a sewage effluent or a rainwater rinse. On the other hand, echovirus 6 showed significantly less soil affinity, with 78% of the virus adsorbing. The viruses were mobilized with either a sewage effluent or a rainwater rinse.

Adsorption and mobilization of mutant LSc strains. Since the adsorption-elution pattern of poliovirus LSc 2ab was different from that of all the other enteroviruses tested, it was of interest to determine whether mutant forms of this virus had altered soil affinities. Two independently isolated guanidine-resistant mutants of poliovirus type 1 LSc 2ab were analyzed for their ability to adsorb and be eluted by rainwater from natural cores. Table 8 illustrates the effect of guanidine, an inhibitor of viral protein synthesis (19), on the replication of poliovirus LSc 2ab. The EOP of the sensitive reference strain was extremely low, whereas both resistant strains (T and B) replicated well in the presence of 100 μg of guanidine per ml. The behavior of the guanidine-sensitive and -resistant strains markedly differed on soil cores (Table 9). Poliovirus LSc 2ab adsorbed well to soil cores (93%), and the adsorbed viruses were not affected by either a sewage or a rainwater rinse. Although the adsorption and sewage elution patterns of the guanidine-resistant B strain were similar to those of the sensitive strain, the rainwater rinse resulted in an elevated amount of viruses in the core filtrate. The guanidine-resistant T strain of LSc not only demonstrated a reduced adsorptive ability to cores, averaging 72% adsorption, but also showed a significantly greater amount of virus in the sewage filtrate. About 37% of the adsorbed viruses were eluted from the soil by using a rainwater rinse.

DISCUSSION

A basic knowledge of the behavior of human enteroviruses in soils is an important consideration in the land application of wastewater. Although the health significance of possible contamination of groundwater by these agents cannot be accurately assessed due to the lack of epidemiological evidence, optimizing land treatment systems for removal of enteroviruses appears to be a reasonable and prudent goal. Until recently, most of the information on virus removal by soils was generated by using a limited number of virus types.

TABLE 6. *Elution of poliovirus type 3 field isolate (V153-1) from cores with artificial rainwater (pH 6.7)*

Elution medium	pH	Conductivity (μmho)	Adsorbed virus (total PFU)	Eluted virus (total PFU)
Sewage effluent	7.34	650	1.36×10^6	6.62×10^3 (0.48)[a]
Rainwater	7.23	800	2.14×10^7	1.43×10^4 (0.06)
	4.30	50	1.43×10^6	3.93×10^5 (27.48)
	6.70	300	2.12×10^7	4.17×10^6 (19.67)

[a] Values in parentheses are percent recoveries of adsorbed viruses.

TABLE 7. *Adsorption and rainwater elution of echovirus types 1 and 6 in soil cores*

Echovirus type	Total PFU recovered from:				
	Input (A)	Unadsorbed (B)	Theoretical adsorbed (A–B)	Sewage filtrate (C)	Rainwater filtrate (D)
1 (Farouk)	1.53×10^5 (100)[a]	0 (0)[a]	1.53×10^5 (100)[a]	—[b]	0 (0)[c]
	1.53×10^5 (100)	1.16×10^3 (0.08)	1.52×10^5 (99.92)	0 (0)[c]	—
6 (D'Amori)	5.31×10^5 (100)	8.75×10^5 (16.48)	4.43×10^4 (83.52)	—	6.71×10^3 (15.14)
	5.31×10^5 (100)	1.46×10^4 (27.50)	3.85×10^4 (72.50)	5.25×10^3 (13.63)	—

[a] Values in parentheses are percent recoveries based on input.
[b] —, No sample.
[c] Values in parentheses are based on the theoretical adsorbed value.

TABLE 8. *Effect of guanidine on the replication of sensitive and resistant strains of poliovirus type 1 LSc 2ab*

Poliovirus strain	PFU/ml		EOP (A ÷ B)
	No guanidine (A)	Guanidine at 100 μg/ml (B)	
LSc 2ab	9.9×10^7	$<1.0 \times 10^3$	$>1.01 \times 10^{-4}$
LSc g'-T	4.3×10^5	4.0×10^5	0.93
LSc g'-B	6.6×10^5	4.30×10^5	0.63

The results obtained in the present study, coupled with the recent findings of Gerba and Goyal (5), indicate that the behavior of enteroviruses in soil is not uniform, and caution should be exercised in extrapolating from data based on a limited number of virus and soil types.

The extent of adsorption and mobilization of viruses in Long Island soils was analyzed by using soil cores (43 by 125 mm) obtained from operating recharge basins. Although these cores have the disadvantage of not allowing resorption of mobilized viruses, they best approximate the ambient soil and recharge conditions. Preliminary data indicated that the clean, unused soil initially used in these studies possessed little virus-removing ability. Conditioning columns with 15 liters of sewage effluent slightly enhanced their virus-removing ability, but there remained appreciably less virus than seen adsorbed to natural cores. Apparently, the buildup of organic matter in the top soil layers was responsible for increased efficiencies (1). Other studies using different soil types (e.g., higher clay and silt content) found efficient virus removal by unconditioned soils (12).

Due to their avirulent nature and ease of handling, attenuated strains of poliovirus have been used extensively in soil adsorption studies. Virulent poliovirus type 2 Lansing has also been used in such experiments (9). Analysis of the data has indicated that these viruses adsorb extremely well to a wide variety of soils. Recent evidence by Gerba and Goyal (5) indicated that both reference and field strains of all three types of poliovirus adsorbed well to Flushing Meadow soils. Data presented in this paper demonstrate the ability of a variety of polioviruses, including field and reference strains, to adsorb to a highly permeable, coarse, sandy soil that would generally be considered a poor virus-absorbing soil (2). In studies which have demonstrated poor poliovirus adsorption to soils, additional factors such as high infiltration rates (9) and experimental adsorbing conditions (e.g., autoclaved soil and sewage effluent) (18) may have been responsible for alterations in soil-virus affinities.

Viruses adsorbed to soils are not irreversibly bound and may be desorbed by percolating fluids under certain conditions. Since adsorption is basically an electrostatic attraction (2), changes in the degree of electronegativity of virus and soil particles by changes in the pH or ionic strength of the percolating fluids can result in desorption and mobilization of the virus. The movement of soil-bound polioviruses by distilled water has been demonstrated (3, 6, 13). Maximum virus elution from small cores has been observed to coincide with the minimum conductivity levels of column effluents (3). The present results have shown the mobilization of certain types and strains of polioviruses (but not others) by use of an artificial rainwater. The rainwater, which reflects the anion, cation, and pH characteristics of a typical northeastern United States rainfall (4), is more representative of true rainfall than is distilled water. Rainwater rinses led to a significant mobilization of a number of polioviruses, including a type 3 reference strain (Leon) and types 1 and 3 field isolates. Total adsorbed viruses eluted by a 200-ml rinse ranged from 24 to 66%. Duboise et al. (3) had previously observed that 16% of strain Chat was eluted from natural cores by one rinse with distilled water and that 22% was eluted with three rinses. Soil-bound strain LSc showed no significant movement (2.83%) after an application of rainwater. Several studies have also reported limited movement of LSc by application of distilled water in columns (250 by 10 cm) (6, 13). Mobilized viruses were found to resorb lower in the column, and few were detected in the outflow. Due to the size constraints of the cores used in the present experiments, we cannot comment on the resorption ability of any of the viruses. However, based upon the amount of viruses mobilized by rainwater, we feel that they would move

TABLE 9. *Adsorption-elution profiles of guanidine-sensitive and -resistant strains of poliovirus LSc 2ab*

Poliovirus strain	Total virus recovery (%) as:		
	Theoretical adsorbed[a] (A)	Sewage filtrate[b] (B)	Rainwater filtrate[b] (C)
LSc 2ab	91.82	—[c]	0.46
	95.67	<0.44	—
LSc g'-B	71.33	0.00	38.63
	96.51	0.87	6.62
LSc g'-T	68.55	4.07	59.73
	76.50	9.49	15.54

[a] Calculated by subtracting unadsorbed viruses from the input.

[b] Based on the theoretical adsorbed.

[c] —, No sample.

in greater numbers and to greater depths in the soil. It is also important to note that one strain (V234-1) was desorbed in significant quantities (14%) by sewage effluent. These data appear to indicate that although polioviruses can easily adsorb to most soils, there are type and strain differences that allow some (e.g., type 3 field isolates) to be more easily desorbed and mobilized by low-ionic-strength substances. Conductivity appears to be a more important mobilizing factor than pH in this system, since both acid- and near-neutral-pH rainwater rinses of low ionic strength were found to effectively elute viruses.

Although variable elution behavior has not been previously demonstrated in soils, it has been observed in a number of other column types that were used in attempts to differentiate between virulent and avirulent viruses. Woods and Robbins (24) observed that although attenuated LSc and virulent Mahoney strains adsorbed equally well to aluminum hydroxide gels, only the Mahoney strain could be efficiently eluted with a pH 7 phosphate buffer. A similar pattern was observed on diethylaminoethyl-cellulose columns for the same viruses (8). Kona (11), using calcium phosphate columns, demonstrated a similarly varied elution profile for virulent Brunhilde and Mahoney strains and the attenuated LSc strain. Both virulent strains could be eluted with 0.01 to 0.05 M phosphate buffer, whereas LSc required 0.15 to 0.25 M buffer for elution. In the present study, two field isolates tentatively identified as wild poliovirus by temperature marker analysis were easily eluted from soil cores by rainwater, whereas avirulent strain LSc was not. However, since an avirulent field isolate (V234-1) was also mobilized by rainwater, a distinguishing elution pattern between virulent and avirulent poliovirus could not be established. Elution appears to be dependent on the virus type and strain.

Evidence that varied electronegative charge strengths are responsible for differing adsorption-elution patterns of viruses, even within a strain, has been recently presented by Lance and Gerba (12). The authors observed that the vertical distribution of LSc in a soil column (250 by 10 cm) was essentially unchanged over a wide concentration of applied viruses. Although a majority of viruses adsorbed in the top 5 cm of soil, others were found to move to greater depths. The authors suggested that a slight variation of charge strength within a population of viruses was responsible, with those having stronger negative charge adsorbing immediately and a subpopulation, with a somewhat reduced negative charge, moving further down the column. The variations in charge strength appear to be minimized by adding divalent cations or by drying the soil column (6, 13). In addition, soils having a pH of 5.0 and below best adsorbed a wide variety of enteroviruses, and the low soil pH may minimize the effect of charge differences (6). Duboise et al. (3) maintained that viruses adsorbed further from the surface of a soil column would more easily be mobilized due to a decreasing ionic gradient within the column. Ions from percolating fluids would be adsorbed by the surface soils until they reached a point of saturation. Fewer ions would then be available for adsorption at greater depths. Thus, the viruses initially adsorbed at greater depths may have a weaker charge, as well as a less optimal adsorbing environment, and be easily influenced by drastic changes in pH and ionic strength.

Strain differences have been recently proposed by Gerba and Goyal (5) to account for the variability in soil adsorption of certain echo- and coxsackieviruses. Elution variability was not studied. Our results with coxsackievirus type B3 and echovirus types 1 and 6 lend support to their observations. Since viral capsid proteins differ slightly among types, strains, and substrains, a somewhat different isoelectric point can be expected, and thus a different electronegative charge under the same conditions.

Analyzing the data from this and other reports, we have concluded that the adsorption and elution behavior of poliovirus LSc in soil is different from that of a number of other enteroviruses. However, researchers are limited to studying this and other avirulent enteroviruses in all field and many laboratory experiments. We therefore sought to derive a mutant LSc that retained its avirulent nature but had altered capsid properties, resulting in altered adsorption-elution characteristics. Such a virus may more closely resemble other enterovirus-soil interactions. The results presented in this paper appear to represent such an option. Two independently isolated guanidine-resistant mutants were analyzed and found to have adsorption-elution behaviors different from those of the parent strains. Strain B resembled the parental LSc strain in that it was not significantly mobilized by sewage. However, it demonstrated a significant elution with rainwater. Strain T also differed from the parent strain, demonstrating a significantly different sewage and rainwater elution profile. Some variability in the adsorption-elution pattern within the experimental runs was observed and may have been due to a mixed subspecies population within the strain. Although the strains were plaque purified three times, the EOP in 100 μg of guanidine per ml was less than that in the absence of guanidine, possibly indicating the presence of a residual

guanidine-sensitive population. Extensive purification may increase the EOP. Alterations in the capsid proteins of the guanidine-resistant strains will be investigated to determine the cause of the varied adsorption-elution behavior of these viruses.

In summary, we observed the efficient adsorption of a number of polioviruses to a highly permeable, coarse sandy soil. The elution of the viruses by sewage effluent or rainwater rinses appeared to be strain dependent, indicating a strain or type variation in the overall charge characteristics of each virus. Poliovirus strain LSc failed to be mobilized by either a sewage or a rainwater rinse, whereas other polioviruses were mobilized by either or both. In addition, two echovirus types demonstrated strain-dependent adsorption-elution behavior. Together, these results suggest that the behavior of poliovirus LSc in soil does not reflect that of a number of enteroviruses, and caution should be exercised in predicting general enterovirus soil models. Two guanidine-resistant strains of poliovirus LSc exhibited elution properties different from those of the parental strain. These avirulent mutant strains may be useful in further laboratory and field studies in predicting the fate of certain enteroviruses.

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